AD_____

GENETIC AND SEROLOGICAL ANALYSIS OF SALMONELLA-COLI PHAGE HYBRIDS AND DEVELOPMENT OF NEW MUTATOR PHAGES WITH EXPANDED HOST RANGES

ANNUAL REPORT

NOBUTO YAMAMOTO

NOVEMBER 1985

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-85-G-5002

Hahnemann University School of Medicine
Philadelphia, Pennsylvania 19102

Approved for public release; distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO. / 3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle): Genetic and Serological Analysis of Salmonella-coli Phage Hybrids and Development of New Mutator Phages with Expanded Host Ranges	5. TYPE OF REPORT & PERIOD COVERED: Annual Report (11/1/84 to 10/31/85) / 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Nobuto Yamamoto	8. CONTRACT OR GRANT NUMBER(s): DAMD 17-85-G-5002
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Hahnemann University School of Medicine, Broad and Vine Street, Philadelphia, PA 19102	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 61102A, 3M161102B10-RE-062
11. CONTROLLING OFFICE NAME AND ADDRESS: U.S. Army Research and Development Command, Fort Detrick, Frederick, MD 21701-5012	12. REPORT DATE: November 1985 / 13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report): Unclassified / 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

High specialized transducing phage; Mutator phage; Recombination Tail fiber; Host range; Tail antigen; Serological neutralization; Invertible segment

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

We have isolated hybrid phages between the evolutionary distant phages Salmonella phage P22 and E. coli mutator phage Mu. The MuimmP22 hybrid class carries the entire late genes of Mu and some early genes, at least att-c-12 regions, of P22. MuimmP22 hybrid class is divided into two types, MuimmP22 and MuimmP22dis, with respect to their immunity pattern. P22 infects and grows in strains lysogenic for MuimmP22 though it carries the c region of P22. MuimmP22dis is able to grow in MuimmP22 lysogens, →

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

indicating that MuimmP22dis type carries second immunity region (immI) of P22. Indeed strains lysogenic for MuimmP22 is immune to P22. MuimmP22dis confers the hosts for 0-1 antigen conversion because of the inheritance of the a1 gene which is situated between immI and c region of P22.

Both MuimmP22 and MuimmP22dis types are unable to infect E. coli strains K12 and C while they infect smooth derivatives of E. coli - S. typhimurium WR4028. This observation suggests that the G(+) segment for the Mu tail fiber region is inverted in the MuimmP22 hybrid class and transcribed in inverse G(-) orientation. This hypothesis is supported by a reduced neutralizing activity of an anti-Mu serum.

Furthermore, P22immMu hybrid class containing the entire late genes of P22 and some early genes at least the c region of Mu has been isolated. P22immMuc+ is, however, unable to establish stable lysogens.

SUMMARY

Hybrids between Salmonella phage P22 and coli mutator phage Mu have been isolated, using their common hosts E. coli - S. typhimurium recombinants. Although these evolutionary distant phages P22 and Mu have no genetic homology and totally different gene organizations and genomic structures, hybrids were found at an extremely low frequency (10^{-12} or less). MuimmP22 hybrid class carries the entire late genes of Mu and some early genes at least the c region of P22. This hybrid class is subdivided into two types, MuimmP22 and MuimmP22dis by their immunity pattern. P22 grows in strains lysogenic for MuimmP22 though it carries the c region of P22. MuimmP22dis is able to grow in MuimmP22 lysogens, indicating that MuimmP22dis carries the second immunity (immI) region of P22. In fact strains lysogenic for MuimmP22dis are immune to P22 infection.

Mapping analysis of the hybrids by backcrosses with various P22 derivatives revealed that MuimmP22 carries at least the att-c-12 segemnt of P22 at the right terminal of the hybrid genome. In addition MuimmP22dis carries the antigen conversion gene a1 which is situated between ImmI and c genes of P22. Both MuimmP22 and MuimmP22dis types are unable to infect E. coli strains K12 and C while they infect smooth derivatives of E. coli - S. typhimurium WR4028. This observation suggests that the G(+) segment for the Mu tail fiber region is inverted in the MuimmP22 hybrid class and transcribed in inverse G(-) orientation. This hypothesis is supported by a reduced neutralizing activity of an anti-Mu serum. These hybrid phages were however unable to induce mutations in the host cells. Therefore, the c and transposase A (and B) genes and/or the inverted termini of Mu have beem lost during the process of hybrid formation.

Furthermore P22immMu hybrid class containing the entire late genes of P22 and some early genes at least the c region of Mu has been isolated. P22immMuc+ is, however, unable to establish stable lysogeny.

Though we initially planned for development of a gene cloning vector, we have not established a recombinant DNA method for these hybrid phages during this period.

CONTENTS

PROGRESS

1. Development of isolation procedures for hybrids between Salmonella phage P22 and coli mutators phage Mu.

A smooth E. coli - S. typhimurium recombinant strain WR4028 is a sensitivie host for P22 but resistant to Mu whereas a rough recombinant strain WR4027 is a sensitive host for Mu but resistant to P22. A P22-resistant deriviative of WR4028, WR4028/22, is also sensitive to Mu phage. Thus WR2027 and WR4028/22 provided hosts for establishing Mu lysogeny. Since these WR4028/22(Mu) and WR4027(Mu) lysogens are rough and resistant to P22, their superinfection with a mixture of rough specific pahges (R) led us to isolate smooth revertants designated as NY4028(Mu) and NY4027(Mu) respectively. These smooth Mu lysogens were used for propagation of P22 phage and interaction with Mu phage. The rough Mu lysogens, WR4028/22(Mu) and WR4027(Mu) were used for plating P22 lysates previously grown in the smooth Mu lysogens to select hybrids between P22 and Mu phages.

2. Isolation of hybrids between P22 and Mu.

Although P22 and Mu have no genetic homology and totally different chromosomal gene organizations, we were able to isolate MuimmP22 hybrid class at an extremely low frequency (10^{-12} or less). To our surprise MuimmP22 hybrid class is able to infect smooth hosts such as WR4028.

These hybrids have not been found in P22 stocks previously grown in recA strains lysogenic for Mu phages. The recA dependent mechanism and extremely low frequency of hybrid formation suggest that crossovers between P22 and Mu occurs through small accidental homologies rather than non-homologous transposition.

3. Immunity response of MuimmP22 hybrid class.

MuimmP22 hybrids so far isolated are divided into two types, MuimmP22 and MuimmP22dis by their immunity pattern. P22 can grow in strains lysogenic for MuimmP22 though the latter carries the c region of P22. MuimmP22dis is able to grow in MuimmP22 lysogens, indicating that MuimmP22dis type carries the second immunity (immI) region in addition to the c region of P22. In fact, strains lysogenic for MuimmP22dis are immune to P22 infection.

4. Mapping of MuimmP22 hybrids.

To approximate the length of P22 DNA in MuimmP22 hybrid class which is homologous to P22, backcrosses were performed between these MuimmP22 hybrids and P22 deriviaties. Since P22 can infect WR4028(MuimmP22) and induce the prophage, backcross P22 recombinants can be scored for mapping the homologous region of these phages.

In superinfection of WR4028(MuimmP22) with P22c2ts12, the total frequency of P22 recombinants was approximately 1%. P22 recombinants able to replicate at 40°C, exhibiting the wild type ts+ phenotype, were scores for the presence of c+ or c2 phenotype. Of 887 P22 recombinants with the

ts+ phenotype, 445 also obtained c+ phenotype (turbid plaques), while 442 retained the c2 phenotype (class plaques). The ratio of 445:442= 1:1 indicates that the left hand homology stretch from the c2 gene extends a length equal to the distance between c2 and 12 genes. When we plated the P22 superinfected lysates at 30°C , 243 P22 recombinants expressed the c+ phenotype were cloned. Each of these clones were tested for their ability to replicate at 40°C, with appropriate 30°C controls to determine how many had acquired the gene 12 wild type (ts+) phenotype. A total of 20 clones retained ts12 phenotype while the remaining 233 exhibited the ts+ phenotype. The ratio of 20: 223= 1:11 of these recombinants suggest that the right homology stretch from the gene 12 is 11 times of the length between c2 and 12 genes. However backcross analysis of MuimmP22 with various P22 amber mutants revealed that P22 genes from the right of the 12 gene are not present in MuimmP22. The high frequency of P22c+ts+ recombinants is therefore due to a consequence of single crossovers suggesting that the right terminal of the hybrid genome ends with P22 homology at the adjacent to the gene 12. Acquisition of the P22 segment at the right arm of the hybrid has resulted in loss of the c-A-B segment at the left of Mu phage. Therefore, the gene organization and genomic structure of the MuimmP22 hybrid class is very similar to that of coliphage P2.

5. The antigen conversion gene a1 in MuimmP22dis.

Since the somatic antigen conversion gene a1 is located between the c and immI of P22, MuimmP22dis should carry the a1 gene. Two smooth E. coli - S. typhimurium strains WR4028 and WR4028E were lysogenized with MuimmP22 and with MuimmP22dis and tested for antigen conversion in slide agglutination test using single factor 0-1 antiserum. MuimmP22dis conferred smooth hosts 0-1 antigen conversion while MuimmP22 did not.

6. Host range and tail fiber antigenecity of MuimmP22 hybrid class.

Although the entire late genes of MuimmP22 hybrid class are derived from those of Mu, both MuimmP22 and MuimmP22dis hybrids are unable to form plaques on E. coli strains K12 and C. However, they infect smooth E. coli - S. typhimurium recombinant strains WR4028 and WR4028E. This observation suggests that the G(+) segment for the Mu tail fiber region is inverted in MuimmP22 hybrid class and transcribed in inverse (-) orientation. Changes in amino acid sequence should change not only host ranges but also antigenicity of the tail fiber. MuimmP22 and MuimmP22dis phages were tested for plaque neutralization with an anti-Mu having k value of about 50 for MuG(+) phage, kindly supplied by Dr. Martha Howe. Slow neutralization (5-fold in 1 hr) of plaque forming ability was observed with a 10-fold diluted serum. This observation supports the hypothesis that the G(+) segemnt for the Mu tail fiber regions is inverted as G(-) orientation in MuimmP22 hybrid class.

7. Lack of mutator activity of Muimm P22 hybrid class.

Mutagenesis by these hybrid phages were tested with maltose, xylose and galactose genes. WR4028 or WR4028E were infected with MuimmP22 or MuimmP22dis and plated on McConkey or EMB agar supplemented with maltose, xylose or galaclose at 30° or 37°. No mutations were detected. This observation suggests that the transposase A and B genes and/or inverted repeat termini of Mu is replaced with the att-c-12 genes of P22 in MuimmP22

hybrid class. However addition of new inverted sequences, having affinity to 12 and 18 gene products to the termini of the hybrid genomes may create transposable hybrid phages.

8. Isolation of P22immMu hybrid class

A high titer stock of P22c2ts12 was UV irradiated for 60 sec (1,200 ergs/mm^2) and plated on Mu lysogens at a permissive temperature to obtain confluent lysis plates. When phage stocks extracted from the confluent lysis plates were plated on smooth E. coli - S. typhimurium WR4028 or S. typhimurium Q at a nonpermissive temperature, faint turbid plaques were detected. These faint turbid plaque formers were designated as P22immMu hybrid class because they carry the entire late genes of P22 and some early genes at least the c region and also probably transposase A and B genes of Mu. P22immMuc+ is, however, unable to form stable lysogeny of the hosts.

Publication:

Yamamoto, N., Droffner, M.L., Yamamoto, S., Gemski, P. and Baron, L.S. High frequency transduction by phage hybrids between coliphage Ø80 and Salmonella phage P22. J. Gen. Virol. 66, 1661-1667 (1985).